Molecular Biology

IN SEARCH OF CHROMOSOMAL FUSIONS IN TELOMERIC MUTANTS OF *Tetrahymena thermophila*

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The ends of eukaryotic chromosomes are protected by nucleoprotein structures called telomeres. The DNA component of a telomere consists of repeating GC-rich sequences with the protein complexes bound to it. Studies have shown that mutated telomeres are detrimental to the cell, causing double stranded breaks, non-homologous end-joining, chromosomal fusions and increased genomic instability. Previous research in our lab on the single celled eukaryote, *Tetrahymena thermophila,* has shown a severe anaphase arrest in response to telomeric DNA mutations. Further analysis using Southern blot suggests extensive loss of telomere sequences in these mutants. In other eukaryotes, such telomere loss results in chromosomal fusions. Therefore, in our telomeric mutants we hypothesize the anaphase arrest is due to chromosomal fusions. In order to test our hypothesis, we developed a Polymerase Chain Reaction (PCR) assay that can detect telomere fusions. Primers were designed from the sequences just adjacent to the telomere of *T.thermophila* called telomere-associated sequences (TAS). We designed a positive control to ensure that our assay could overcome the typical difficulties encountered in amplifying long GC-rich PCR products. TAS clones of *T.thermophila* were cut and ligated to mimic potential *in vivo* fusion events. PCR on our positive control amplified a fusion product of ~850 base pairs. Preliminary assays of the mutant DNA did not amplify any fusion products. These findings suggest any of three possibilities: 1) the absence of chromosomal fusions, 2) a small percentage of fusions below the sensitivity range of the assay, or 3) the fusion product is beyond the size limit of the assay. Further research is being conducted to extend the existing size limit of the PCR assay to allow for the amplification of larger fusion products.